

I claim:

1. An attenuated derivative of a pathogenic microorganism with a cell wall comprising diaminopimelic acid (DAP) which further comprises:

- 5
- (a) an inactivating mutation in a native chromosomal essential gene encoding an essential enzyme which catalyzes a step in the biosynthesis of DAP;
- (b) a recombinant complementing gene on an extrachromosomal vector encoding a functional replacement for the essential enzyme, wherein the complementing gene can recombine to replace the defective chromosomal gene; and
- (c) a desired gene on the extrachromosomal vector, wherein the desired gene is a
- 10 recombinant gene encoding a desired gene product;
- wherein the desired gene is stably maintained in a progeny population of the microorganism.

2. The microorganism of claim 1, wherein the microorganism is a member of the *Enterobacteriaceae* and the extrachromosomal vector is a plasmid.

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3. The microorganism of claim 2, further comprising an inactivating mutation in a native gene selected from the group consisting of a *pab* gene, a *pur* gene, an *aro* gene, *nadA*, *pncB*, *galE*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *galU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hilA*, *rpoE*, *flgM*, *tonB*, and *slyA*.

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4. The microorganism of claim 3, wherein the desired gene product is an antigen.

5. The microorganism of claim 4, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.

6. The microorganism of claim 2, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapD*, *dapE*, *dapF*, and *asd*.

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7. The microorganism of claim 6, wherein the inactivating mutation is in an *asd* gene and comprises an insertion or a deletion.

9. The microorganism of claim 8, wherein the recombinant complementing gene is an *asd* gene.

10. The microorganism of claim 2, wherein the desired gene is operably linked to a eukaryotic promoter.

11. The microorganism of claim 10, wherein the eukaryotic promoter is a CMV promoter.

12. A recombinant vector comprising a recombinant gene encoding an essential enzyme, wherein the recombinant gene lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence,

5 wherein the vector can functionally replace the essential enzyme when the vector is present in a microorganism having an inactivating mutation in a native gene encoding the essential enzyme.

13. The recombinant vector of claim 12, wherein the vector is a plasmid capable of expressing the essential enzyme in a microorganism that is a member of the *Enterobacteriaceae*.

14. The recombinant vector of claim 12, wherein the essential enzyme catalyzes a step in the biosynthesis of DAP.

15. The recombinant vector of claim 14, wherein the recombinant gene is an *asd* gene.

16. The recombinant vector of claim 12, further encoding a desired gene product.

17. The recombinant vector of claim 16, wherein the desired gene product is an antigen.

18. The recombinant vector of claim 17, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.

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19. The recombinant vector of claim 16, wherein the desired gene product is therapeutic to a vertebrate.

20. The recombinant vector of claim 19, wherein the desired gene product is selected from the group consisting of a lymphokine, a cytokine, and a sperm-specific or egg-specific autoantigen.

21. The recombinant vector of claim 16, wherein the desired gene product is operably linked to a eukaryotic promoter.

22. The recombinant vector of claim 21, wherein the eukaryotic promoter is a CMV promoter.

23. A method of selecting for the presence of a desired gene in a population of microbial cells having an inactivating mutation in a native chromosomal gene encoding an essential enzyme which catalyzes a step in the biosynthesis of DAP, the method comprising

- 5 (a) transfecting the microbial cell with an extrachromosomal vector having
- (i) the desired gene, and
 - (ii) a recombinant complementing gene encoding a functional replacement for the essential enzyme, wherein the complementing gene can recombine to replace the defective chromosomal gene; then
- 10 (b) culturing the microbial cell.

24. The method of claim 23, wherein the microbial cell is a member of the *Enterobacteriaceae*.

25. The method of claim 24, wherein the microbial cell further comprises an inactivating mutation in a native gene selected from the group consisting of *aroA*, *aroC*, *aroD*, *cdt*, *cya*, *crp*, *phoP*, *phoQ*, *ompR*, *galE*, and *htrA*.

26. The method of claim 24, wherein the desired gene product is an antigen.

27. The method of claim 26, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.

28. The method of claim 23, wherein the desired gene product is selected from the group consisting of a lymphokine, a cytokine, and a sperm-specific or egg-specific autoantigen.

29. The method of claim 23, wherein the desired gene is operably linked to a eukaryotic promoter.

30. A vaccine for immunization of a vertebrate, the vaccine comprising live bacterial cells of an attenuated derivative of a pathogenic bacterium in a pharmaceutical carrier, the bacterial cells comprising:

- 5 (a) an inactivating mutation in a native chromosomal essential gene encoding an essential enzyme which catalyzes a step in the biosynthesis of DAP;
 - (b) a recombinant complementing gene on an extrachromosomal vector encoding a functional replacement for the essential enzyme, wherein the complementing gene can recombine to replace the defective chromosomal gene; and
 - 10 (c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;
- wherein the desired gene is stably maintained in a progeny population of the microorganism.

31. The vaccine of claim 30, wherein the inactivating mutation is in an *asd* gene and comprises a deletion or an insertion.

32. The vaccine of claim 30, wherein the bacterium further comprises an inactivating mutation in a native gene selected from the group consisting of *aroA*, *aroC*, *aroD*, *cdt*, *cya*, *crp*, *phoP*, *phoQ*, *ompR*, *galE*, and *htrA*.

33. The vaccine of claim 30, wherein the desired gene product is an antigen.

34. The vaccine of claim 33, wherein the antigen is selected from the group consisting of a bacterial antigen, a viral antigen, a fungal antigen, a parasitic antigen, a gamete-specific antigen, an allergen, and a tumor antigen.

35. The vaccine of claim 30, wherein the recombinant complementing gene on an extrachromosomal vector lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence.

36. The vaccine of claim 30, wherein the desired gene is operably linked to a eukaryotic promoter.

37. A method of inducing immunoprotection in a vertebrate, comprising administering to the vertebrate the vaccine of claim 30.

38. A method of delivering a desired gene product to a vertebrate, the method comprising administering to the vertebrate live bacterial cells of an attenuated derivative of a pathogenic bacterium, the bacterial cells comprising:

- 5 (a) an inactivating mutation in a native chromosomal essential gene encoding an essential enzyme which catalyzes a step in the biosynthesis of DAP;
 - (b) a recombinant complementing gene on an extrachromosomal vector encoding a functional replacement for the essential enzyme, wherein the complementing gene can recombine to replace the defective chromosomal gene; and
 - 10 (c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding the desired gene product;
- wherein the desired gene is stably maintained in a progeny population of the microorganism.

39. The method of claim 38, wherein the bacterium is an *Enterobacteriaceae* and the bacterium further comprises an inactivating mutation in a native gene selected from the group consisting of *aroA*, *aroC*, *aroD*, *cdt*, *cya*, *crp*, *phoP*, *phoQ*, *ompR*, *galE*, and *htrA*.

40. The method of claim 38, wherein the essential gene is selected from the group consisting of *dapA*, *dapB*, *dapD*, *dapE*, *dapF*, and *asd*.

41. The method of claim 38, wherein the desired gene product is an antigen.

42. The method of claim 38, wherein the desired gene product is therapeutic to the vertebrate.

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